**2102-Pos Board B239**
**Multi-Scale Linkages between Single-Molecule Integrin Dynamics and Cell Protrusion**
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Recent advances in light microscopy permit the development of cyto-architectural blueprints with single-molecule resolution. It is now conceptually possible to relate the organization of the molecular machinery to cellular function. However, the disparity between the spatial and temporal scales of molecular and cellular behaviors poses substantial challenges in deriving these relationships. New approaches are required to integrate discrete single-molecule behavior with continuous cellular-level processes. Here, we combined intercalated molecular and cellular imaging with an analytical framework to reveal the roles that individual integrin molecules play in the process of initiating cell adhesion and migration. Despite the stochasticity of molecular and cellular behaviors, our data uncovered 'rules' of molecular organization for the components of a functional nascent adhesion, namely $\alpha_v\beta_3$-integrin, talin and actin, as well as the relationship between the local molecular organization of integrins and cell edge movement. We found that integrins exhibit a basal spatial gradient, with highest density and slowest mobility at the cell edge. In preparation for protrusion, this gradient transiently and locally increases, indicative of the initiation of nascent adhesions prior to cell edge protrusion. Moreover, the molecular behavior of talin, actin and $\beta_3$ integrin mutants revealed that talin binding regulates integrin mobility, while integrin density modulation is primarily dependent on actin. These data establish a multi-scale linkage between characteristic variations in discrete stochastic molecular behaviors and continuum cellular behaviors.

**2103-Pos Board B240**
**Weak Ergodicity Breaking of Membrane Receptor Motion Stemming from Random Diffusivity**
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The application of techniques such as fluorescence correlation spectroscopy and single particle tracking to the plasma membrane of living cells continuously evidences subdiffusive motion of proteins and lipids. This anomalous motion is generally associated to the interplay of molecular crowding, diffusion barriers and specific interactions. Addressing the cause of subdiffusion is essential for understanding molecular mechanisms underlying cellular function, such as target search, kinetics of transport-limited reactions, trafficking and signalling. Recently, the subdiffusive motion of some cellular and membrane components has been associated to weak ergodicity breaking and attributed to transient immobilization caused by interactions with microtubules or actin cytoskeleton. These works have opened new questions about the role of nonergodic subdiffusion in the dynamics of living systems.
By means of single particle tracking experiments, we show that the dynamics of DC-SIGN, a transmembrane receptor with unique pathogen recognition capabilities, also reveals subdiffusion with signatures of weak ergodicity breaking and aging. In contrast to other biological systems, we demonstrate that DC-SIGN nonergodic subdiffusion cannot be explained by transient immobilization, but rather is compatible with dynamical heterogeneity induced by spatiotemporal changes of diffusivity. While nonergodic diffusion due to long-lived immobilization events can be understood within the framework of continuous-time random walk, our experimental data are accurately interpreted through a new theoretical model describing anomalous transport in biological systems and complex media. A comprehensive analysis of mutated forms of the receptor allowed us to establish a link between receptor molecular structure, nonergodic diffusion and function. Our results underscore the role of spatiotemporal disorder in the dynamics of cell membrane receptors. In addition, they have broad implications to other heterogeneous systems where the occurrence of nonergodicity remains unexplored.

**2104-Pos Board B241**
**Restricted Mobility of TonB and FepA in E. coli Membranes**
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Nutrient uptake in Escherichia coli requires transport across the cell's lipopolysaccharide-rich outer membrane, passage through the peptidoglycan layer containing periplasmic space and finally transport across the cell's inner cytoplasmic membrane. In many cases, the first step in this process involves transit through a class of beta-barrel proteins in the outer membrane known as TonB-dependent transporters (TBDTs). This uptake requires interaction between the TBDTs and the cytoplasmic membrane protein, TonB. TonB, in complex with ExbB and ExbD, is thought to derive energy from proton-motive force of the cytoplasmic membrane to be transduced to the outer membrane TBDTs for transport. Here, we have investigated the mobility of TonB in the inner membrane and FepA, a TBDT responsible for transport of the iron chelating siderophore enterobactin (FeEnt), in the outer membrane of E. coli at the single molecule level. We find that FepA is located throughout the outer membrane while TonB is found excluded from the inner membrane's polar regions. Both proteins are observed to undergo free diffusion in the absence of FeEnt within confining domains in their respective membranes (a domain radius of 130 nm for FepA and 240 nm for TonB). Effects of depolarization of the inner membrane, deletion of ExbB/D and presence of FeEnt will be discussed in term of interactions between the proteins.

**2105-Pos Board B242**
**Cryo-Electron Tomography and Computer Simulations Reveal Distinct CheA Kinase Conformation in Bacterial Chemotaxis Signaling Receptor Complex**
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Improved sample preparation, data collection, and image processing pipelines have ushered in a new era in cryo-Electron Tomography (CryoET). Electron density maps of large, conformationally heterogeneous assemblies can be determined at subnanometer resolutions and coupled with high resolution structural information and large scale molecular dynamics simulation to gain atomistic understanding of large and complex cellular machinery that is otherwise unattainable. We use such an integrative approach to investigate the signal transduction in bacterial chemotaxis arrays - large transmembrane protein complex assemblies responsible for modulating the motility of bacteria. These arrays are responsible, in part for appropriately biasing the cell's inherent random walk, and also retaining the memory of recent states of the system. Despite recent advances, fundamental questions on how the components of the system work together to transduce the signal from membrane receptor to kinase molecule, and how the individual signaling unit works cohesively to achieve a large gain in signaling remain unanswered. We devised a novel *in vitro* reconstitution system to build extended signaling arrays on a lipid monolayer using purified protein components, providing an unprecedented number of signaling units in a thin layer (~150-200nm) ideal for cryoET. The individual subvolumes from 3D tomograms are extracted, aligned, classified and averaged, resulting in a 3D density map of the basic chemotaxis signaling unit comprising the chemoreceptor, the histidine kinase CheA and the cofactor CheW. The density map reveals an unexpected asymmetry among three kinase CheA dimers, indicating distinct CheA conformations within the unit. Large scale all-atom molecular dynamics simulations further point to the specific side chain contacts that mediate the transition of CheA conformations, which we show, through biochemical assays, to fully abrogate chemotactic function if disrupted.

**2106-Pos Board B243**
**Tracking Ceacam1 Interactions and Dynamics with homo-FRET and Image Correlation Techniques**
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The carcinoembryonic antigen (CEA) family plays an important role in numerous normal and pathogenic processes related to cellular growth and differentiation. Crucial roles of various CEA-family members are well established while the effect of carcinoembryonic antigen-related cellular adhesion molecule 1 (CEACAM1) on immune-cell function has only recently been recognized. CEACAM1 is known to exist in both monomeric and dimeric states that are heterogeneously distributed at the cell surface. However, which form participates in cell-cell interactions and its related dynamics remain unknown. We report here details of CEACAM1's spatial distribution, oligomeric state, diffusional characteristics and dynamics as determined by live cell homo-fluorescence energy resonance transfer (homo-FRET) microscopy and imaging total internal reflection fluorescence correlation spectroscopy (ITIR-FCS).